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Genetic Divergence between Wild and Cultivated Pearl Millets (*Pennisetum typhoides*)

I. Male Sterility

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With one figure and 4 tables

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Abstract

In two crosses between wild and cultivated pearl millets nucleo-cytoplasmic male sterile plants have been created by transfer of a cultivated genotype into a wild cytoplasm. The phenomenon is not reciprocal: the wild genotype placed in cultivated cytoplasm maintains an excellent male fertility. A completely male sterile BC 7 line in wild cytoplasm background greatly resembling the cultivated parent, has been constructed from one of the two crosses. The genetics of its restoration seems to imply three independent genes (2 major and 1 minor). Restoration tests of this new male sterile line indicate that it is different from the three known male sterile sources A1, A2 and A3. Wild forms seem to possess a higher frequency of restorer alleles than cultivated forms, regardless of their geographical origins.

Key words: *Pennisetum typhoides* — biosystematics — wild ecotypes — cultivars — genetic divergence — male sterility — fertility restoration

The genetic relationships between wild and cultivated pearl millets have been little investigated. The classical botanists (STAPF, HUBBARD, HUTCHINSON) distinguished a multitude of botanical species in annual cultivated and wild pearl millets. In a study of two wild × cultivated crosses, BILQUEZ and LECOMTE (1969) concluded that there was a single biological species. Observing the excellent fertility of three wild × cultivated hybrids, BRUNKEN (1977) also concluded that there was a single biological species, with three botanical subspecies corresponding to cultivated, wild and intermediate types. Another series of wild × cultivated hybrids studied by BELLIARD et al. (1980) and

PERNÈS et al. (1980) did not show any divisions within the biological species; they did define a "domestication syndrome" referring to the type of genetic organization of the characters differentiating wild and cultivated genotypes.

The present study of two wild × cultivated crosses has been undertaken with the long-range aim of evaluating the genetic changes accompanying domestication. We treat more specifically here the question of cytoplasmic effects on fertility.

Materials and Methods

The two crosses studied are *Violaceum* × 'Tiotandé' and B 103 × J 104. Violaceum is a wild ecotype found by BILQUEZ and LECOMTE (1969) in the vicinity of cultivated fields at Bambey in Senegal and studied by him in crosses with two cultivated pearl millets from Senegal and Chad. 'Tiotandé' is a cultivar grown on the Senegal river floodplain during the dry season. The sample was collected by BILQUEZ. This early flowering variety corresponds phenotypically to a type of pearl millet that is widely distributed through Mauritania and Western Mali and is very different from 'Souna', another early flowering pearl millet widely grown in Senegal during the rainy season (MARCHAIS 1982). B 103 is a wild ecotype of *P. mollissimum* collected by MARCHAIS and GROUZIS (1976), 40 km west of Gao (Mali), far from any cultivated area. J 104 is an Indian cultivated line used to make the commercial hybrid HB3 in a cross with the male sterile line 23 A1. Except for J 104, the parents are issued from populations fixed for a morphological type but otherwise genetically heterogeneous.

The first crosses were made in 1978 and since that time, several F2 and back-cross families were made using different parent plants. In parallel with the study of these materials, a process of fixation by selfing the parental sources was undertaken with the view to more precise genetical studies. Most of the plants were grown in O.R.S.T.O.M. greenhouses at Bondy. Some materials were grown in the phytotron greenhouses at Gif sur Yvette under controlled conditions of temperature and humidity (28 °C/24 °C day/night, relative humidity 60 %) which are more favourable to fertility than the conditions prevailing at Bondy.

The nomenclature of the material will be as follows: V and M designate respectively the wild parents *Violaceum* and *Mollissimum*. T and J designate the cultivated parents 'Tiotandé' and J 104. The cytoplasmic background of each genotype is indicated by a small-case letter in front of the name of the generation; e.g. v-F1 (or m-F1) designates the hybrid obtained by the cross V × T (or M × J). t-F1 (or j-F1) designates the reciprocal F1. BC designates back-cross.

The pedigree of selfed plants is described by the list of successive selfed plants (for instance, T5-5-4 is an S2 plant descending from T5).

Results

A. Cross *Violaceum* × 'Tiotande'

1. Observation of reciprocal effects on male fertility

In a first approach, three classes of male fertility were noticed in the different F2 and BC families:

— plants producing an abundant pollen cloud with perfectly dehisced anthers;
— plants producing little pollen with partially and irregularly dehisced anthers;
— plants producing no pollen, with only indehiscent anthers.

Tab. 1 Evaluation of male sterility by the observation of pollen abundance in pooled families at Bondy

Generation	Number of plants at different levels of pollen abundance			Total
	No pollen	Little pollen	Much pollen	
Violaceum 'Tiotandé' lines	0	0	55	55
T 5	0	5	7	12
T 5.5	0	4	7	11
T 5.5.2	0	6	6	12
T 5.5.4	0	0	12	12
T 5.5.7	0	0	10	10
v-F1	0	0	40	40
t-F1	0	0	40	40
v-F2	10	0	214	224
t-F2	2	0	57	59
v-F1 x T	24	0	80	104
T x v-F1	0	0	264	264
T x t-F1	0	3	36	39
t-F1 x T	0	3	13	16

T = 'Tiotandé' V = *Violaceum*

The observed distributions, after pooling the different families of the same generations, are shown in *Table 1*. The most interesting fact is the high frequency of completely male sterile plants in the generation v-F1 × T in comparison with the reciprocal generation T × v-F1, suggesting the existence of a cytoplasmic effect on male sterility. The observed cases of complete or partial sterility in 'Tiotandé' lines or in the generations t-F2, T×t-F1, t-F1×T (in 'Tiotandé' cytoplasm) are most probably dependent on other causes. For example, a totally male sterile t-F2 plant backcrossed to 'Tiotandé' produced an offspring of 6 plants uniformly and perfectly fertile. The reciprocal experiment, performed using totally male sterile plants chosen in the v-F1×T generation (i.e. a backcross in wild cytoplasm) has led to the creation of a totally male sterile line.

2. Fixation of a male sterile 'Tiotandé' line in wild cytoplasm (v-T)

It has been possible to select a male sterile line by successive backcrosses of a chosen male sterile v-F1 × T plant to a 'Tiotandé' line, itself selected for high male fertility. The process is described in *Figure 1*. The BC1 and BC2 generations were observed only at Bondy for the abundance of their pollen cloud. The following backcrosses were observed in parallel at Bondy and at the phytotron at Gif, for the percentage of pollen grains stained by Alexander's reagent (ALEXANDER 1969). With this more severe test, a majority of the BC3 plants had some fertile pollen, in variable proportions. However,

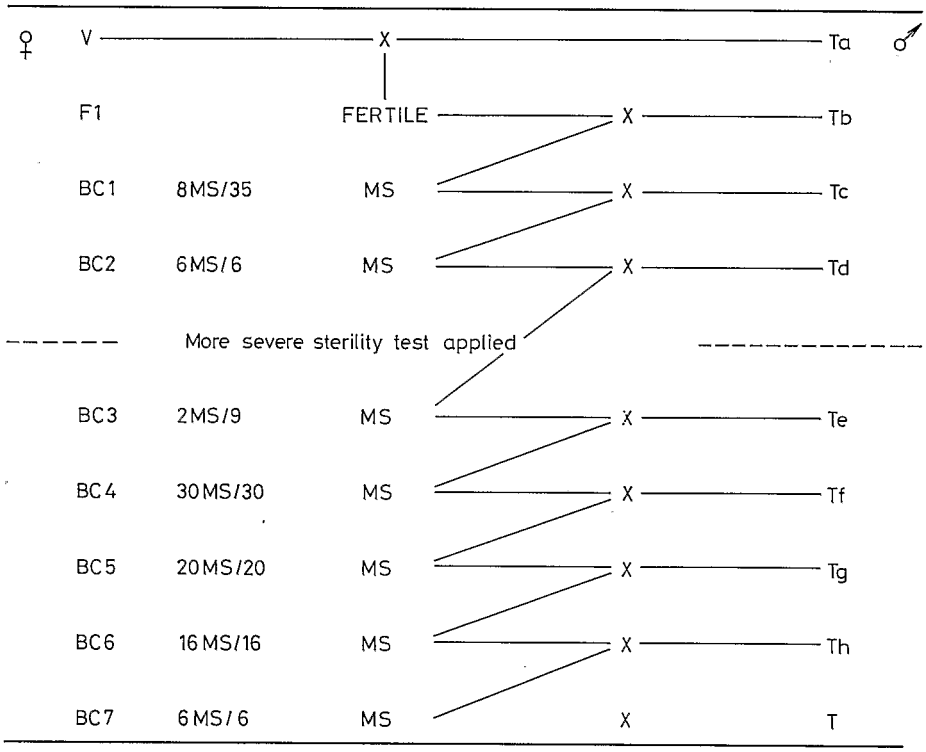

Fig. 1 Creation of a male sterile 'Tiotandé' line (T) by transfer into wild cytoplasm (V). V = *Violaceum* — T = 'Tiotandé' — MS = Male sterile plant. Ta = T5.2 — TB = T5.4 — Tc = T5.5.17 — Td = T5.5.4.9 — Te = T5.5.4.3.2 — Tf = T5.5.4.3.1.5.5 — Tg = T5.5.4.3.1.5.5.4 — Th = T5.5.4.3.1.5.5.4.3

the plant no. 4 of BC3 had dry, indehiscent anthers containing zero per cent stained pollen.

The BC4 generation descending from this plant was composed of 30 plants, all completely male sterile. The fixation of male sterility was confirmed in the 3 next successive backcrosses grown so far. The extreme degree of male sterility obtained is particularly striking given the excellent fertility of the 'Tiotandé' line used as maintainer. The parents T 5.5.4.3, and the next ones all produced after selfing an offspring with abundant pollen stained in a proportion higher than 80 %.

The maternal heredity of the male sterility being established, it is interesting to specify the genetics of its restoration. The data of *Table 1* are insufficient for the following reasons:

— they are collected on genetically heterogeneous material. Non-cytoplasmic sterilities can occasionally occur depending on the family observed;
— notation of pollen abundance is not precise enough to characterize strict male sterility;
— backcrosses to the wild parent using t-F1 are lacking; we do not know the effect of a transfer of the wild genome into cultivated cytoplasm.

Therefore, a complementary study has been carried out by creating a complete series of F1, F2 and BC1 using parents that can reasonably be considered as fixed.

3. Genetics of fertility restoration

Table 2 gives the composition and the pedigrees of the materials studied: the reciprocal character of F1's and backcrosses is perfect or nearly so. Notations of male sterility can be clearly grouped in three classes: S, PF and F.

S: Anthers partially exserted, totally indehiscent. Zero per cent stained pollen grains.

PF: Anthers totally exserted. The degree of dehiscence varies from null to perfect. Very little pollen is released. The percentage of stained pollen varies from zero in indehiscent anthers to 80 % in well dehisced anthers.

F: Anthers perfectly exserted and dehisced. Abundant pollen stained in a percentage higher than 60 %.

All the plants observed in the backcrosses to the wild parent, as well as t-F2, are in the F class, which means that the association of the wild genome with the cultivated cytoplasm maintains a good male fertility.

On the other hand, the cytoplasmic origin of the male sterility observed when associating the cultivated genome with wild cytoplasm is clearly con-

Tab. 2 Material used for determining the genetics of fertility restoration

Generation	Origin	Number of plants at different levels of pollen fertility			Total
		S	PF	F	
Violaceum	(V4 x V6) 1.2.1.2.10	0	0	12	12
t-T 'Tiotandé'	T 5.5.4.3.1.5.5	0	0	12	12
v-T BONDY	BC5 see fig. 1	12	0	0	12
GIF	Idem	8	0	0	8
v-F1	(V4 x V6) 1.2.1.3 x T 5.5.4.3.1	0	0	21	21
t-F1	T 5.5.4.3.1 x (V4 x V6) 1.2.1.3	0	0	21	21
v-F2	(v-F1 above)3	1	1	46	48
t-F2	(t-F1 above) 9	0	0	48	48
T x v-F1	T 5.5.4.3.1.2 x (v-F1 above) 1	0	0	48	48
v-F1 x T BONDY	(v-F1 above) 2 x T 5.5.4.3.1.3	4	4	40	48
GIF	Idem	6	4	32	42
V x t-F1	(V4 x V6) 1.2.6.3 x (t-F1 above) 3	0	0	74	74
t-F1 x V	(t-F1 above) 3 x (V4 x V6) 1.2.6.3	0	0	74	74

Pollen fertility levels:

S Total sterility: no stained pollen

PF Partial pollen fertility: little pollen, variable anther dehiscence, variable percentage of stained pollenfor different anthers.

F Normal pollen fertility: pollen abundant, anthers completely dehiscent, more than 60 % pollen grains stained.

firmed: cases of sterility appear in v-F2 and v-F1 × T, but not in t-F2 and T × v-F1.

Fertility restoration by the wild genes probably takes place on the sporophytic level, for the following reasons:

— the 21 v-F1 plants exhibit a percentage of stained pollen equal to that of the 21 t-F1 plants (i.e. a mean of 90 %, a minimum of 60 % and a maximum of 100 %). In a gametophytic system, v-F1 would have produced a lower amount of fertile pollen than t-F1;

— in a gametophytic system, the degree of partial fertility should be constant for all the anthers of the same spike; this is not the case.

The number of restorer genes and their mode of action can be deduced from the distributions S, PF, F observed in v-F2: 1-1-46 and in v-F1 × T: 10-8-72. Let us call Rf a restorer gene, rf a maintainer gene. In a one-gene model, the wild parent is RfRf, the cultivated parent rfrf. We know v-F1 is F class. We should have in F2 : 1S-3F and in v-F1 × T : 1S-1F. This model is clearly inacceptable. In a two-gene model, the cultivated genotype is $rf_1rf_1rf_2rf_2$ and is sterile in *Violaceum* cytoplasm. We should observe at least one fourth of male sterile plants in v-F1 × T. This 3-1 distribution is very improbable (Chi squared = 9.26, $P < 0.005$). A three-gene model, however, fits the observations very well: Suppose one allele Rf1 or Rf2 alone is able to completely restore male fertility. But Rf3 alone restores fertility only partially, whereas two Rf3 alleles give a complete restoration. In this model,

$v\text{-}rf_1rf_1rf_2rf_2rf_3rf_3$ is S class,
$v\text{-}rf_1rf_1rf_2rf_2Rf_3rf_3$ is PF class,
$v\text{-}rf_1rf_1rf_2rf_2Rf_3Rf_3$ is F class,
$v\text{-}Rf_1rf_1rf_2rf_2rf_3rf_3$ is F class,
$v\text{-}rf_1rf_1Rf_2rf_2rf_3rf_3$ is F class.

The theoretical distribution in F2 is then 1S-2PF-61F, a good fit to the observations (Chi squared = 0.25, P = 0.85). The theoretical distribution in v-F1 × T is 1S-1PF-6F which is also a good fit (Chi squared = 1.38, P = 0.5).

4. Screening for restorer genes

Table 3 gives the fertility observed in crosses between the male sterile line v-T and 35 single pearl millets from diverse geographical and botanical origins. The male plants issued from a homozygous line are presented apart from the male plants sampled from polymorphic populations and wild populations are presented apart from cultivated varieties. This screening provides us with some interesting information:

— The level of restoration is a quantitative character susceptible to furnish a finely graduated scale for measuring genetical divergence between male parents.

— The frequency of restorer alleles seems generally low in cultivated pearl millets, whatever their origin, especially bearing in mind that a single Rf_1 or Rf_2 allele is sufficient for complete restoration. On the contrary, the wild pollinators we used produce a majority of perfectly fertile

Tab. 3 Restoration tests of the 'Tiotandé' male sterile straine v-T Crosses are presented in ascending order of mean fertility. Only one male plant has been crossed for each population tested

Surce of the male plant	Origin	Number of F1 Plants	Percentage of stained pollen		
			Lowest plant	Mean	Highest plant
Cultivated lines					
Massue	Mauritania	4	0	0	0
23 d2 B1	United States	6	0	0	0
75 B3	India	3	0	15	20
J 104	India	6	0	17	40
LIGUI	Chad	4	5	36	65
555-80-2	Souna Senegal	15	60	83	95
Cultivated populations					
2016	Eastern Mali	6	0	0	0
2012	Niger	6	0	2	5
2011	Western Mali	6	0	5	10
215–79	Tanzania	3	0	8	15
3–82	Mauritania	4	0	11	25
2010	Cameroun	6	0	11	50
2013	Souna Senegal	6	0	13	35
2020	Niger	6	0	25	95
P 3265	Togo	4	0	32	75
2014	Sanio Senegal	6	0	35	80
7–82	Zambia	4	20	40	60
558–80	Oasis Niger	4	10	45	70
173–77	Tunisia	15	25	51	90
P 241	Western Mali	4	10	60	80
2019	Niger	3	25	60	80
2015	Western Mali	6	30	62	100
2–82	Algeria	4	35	66	84
C2	Cameroun	15	10	70	95
P6	Cameroun	4	50	70	95
2018	Upper Volta	3	50	72	95
1–82	Morocco	4	50	77	100
2017	Eastern Mali	6	85	95	100
5–82	Tunisia	4	90	95	100
Wild populations					
4–82	Niger	4	5	44	60
B 103	Eastern Mali	12	0	74	99
6–82	Niger	4	30	82	100
476–78	Eastern Mali	3	90	92	95
C1	Cameroun	4	90	95	99

hybrid plants and therefore must possess a higher frequency of restorer alleles. Most of the pollinators used (wild as well as cultivated ones) produce at least one hybrid plant deficient for fertility, which may be indicative of a difference between the *violaceum* cytoplasm and the pollinators' cytoplasms. Indeed, if the occurrence of male sterile plants is not rare in original accessions, all the many crosses made between the male fertile 'Tiotandé' line and other accessions have always shown fully male fertile hybrids.

— The *Violaceum* cytoplasm is likely to be different from the three well-known male sterile cytoplasms A1, A2, A3 (BURTON and ATHWAL 1967). Indeed, a set of inbred lines has been identified that exhibit at Bondy differential restoration abilities in crosses with the male sterile lines: 23d2A1, 239d2A2, 75A3 and v.T. The 'Tiotandé' line, full maintainer of sterility in V cytoplasm, partially restores fertility in A1 and A3 cytoplasms. The Massue line, full maintainer of v-T, fully restores fertility in A1 cytoplasm. The J 104 line gives a high level of male fertility in A3 cytoplasm and a low level in V cytoplasm. 23d2B1, maintainer of v-T, fully restores fertility in A2 cytoplasm.
— No clear geographical distribution of restorer alleles is evident in our present data.

B. Cross Mollissimum × J 104

Qualitatively, cytoplasmic effects on male fertility, very similar to those described in the cross V×T, have been observed in the different generations of the cross M×J. *Table 4* shows the distributions of the S, PF and F classes for male fertility after pooling of all the families for the same generations. No case of S class sterility has been observed but cases of PF class sterility have been noted. They are much more frequent in the m-F2 and m-F1 × J generations than in the reciprocal generations j-F2 and J × m-F1.

The two backcrosses to the wild parent (j-F1 × M, M × J-F1) generated offspring uniformly and perfectly fertile.

Tab. 4 Pooled notations of male sterility in different generations of the cross *Mollissimum* x J 104

Generation	Number of plants at different levels of pollen fertility			Total
	S	PF	F	
M mollissimum	0	0	56	56
J 104	0	0	70	70
m-F1	0	0	33	33
j-F1	0	0	33	33
m-F2	0	40	30	70
j-F2	0	13	52	65
m-F1 x J	0	52	26	78
J x m-F1	0	2	76	78
j-F1 x J	0	2	17	19
J x j-F1	0	4	49	53
j-F1 x M	0	0	30	30
M x j-F1	0	0	30	30
m-BC1 PF x J	0	12	0	12
j-BC1 F x J	0	0	6	6

Fixation of male sterility has been tried by backcrossing a PF class BC1 plant to J 104. The 12 resulting BC2 plants were all PF class, which confirms the cytoplasmic origin of the partial male sterility. Nevertheless, because of the impossibility to obtain S class sterility, this material was abandoned. Let us add that *Mollissimum* cytoplasm seems different not only from J 104 cytoplasm but also from *Violaceum* cytoplasm, for the following reasons:

— A male sterile S class plant appeared in the F1v-T × *Mollissimum* (see *Table 3* — pollinator B 103).

— The PF-F segregations observed in the generations issued from M × J are in disagreement with the genetics of restoration on *Violaceum* cytoplasm.

Discussion

The phenomena of cytoplasmic male sterility reported here demonstrate the existence of genetical differentiation in the pearl millet biological species. Its taxonomical significance is yet to be elucidated. It would be interesting to know if there is a differential geographical distribution of the A1, A2, A3 and V cytoplasms and their restorer alleles.

The restoration ability of wild pearl millets is remarkable, whatever the cytoplasmic background may be: The two wild genotypes V and M maintain a good fertility in the respective cultivated cytoplasms T and J. The five wild pollinators, although having, we presume, a cytoplasm different from V (see the rare cases of deficient restoration), showed a high frequency of good restoration. Additional observations at Bondy have shown that the two wild parents V and M perfectly restore the fertility of the three male sterile cytoplasms A1, A2, A3, a result difficult to obtain with cultivated millets.

Finally, the discovery of the v-T male sterile line opens the way for the creation of new male sterile cytoplasmic sources by crosses between genetically divergent pearl millets.

Zusammenfassung

Genetische Divergenz zwischen Wild- und Kulturformen von Perlhirse (*Pennisetum typhoides*) I. Männliche Sterilität

Aus zwei Kreuzungen zwischen Wild- und Kulturform konnten durch Einlagerung des Genoms der Kulturform in das Plasma der Wildform pollensterile Formen erhalten werden. Im umgekehrten Fall fanden sich voll fertile Pflanzen. Die Restoration der Fertilität scheint von drei Genen abzuhängen. Die Vererbung der Restoration ist unterschiedlich von der der bisher bekannten sterilen Formen A1, A2 und A3. Wildformen scheinen ungeachtet ihrer geographischen Herkunft eine größere Häufigkeit der Restorerallele aufzuweisen als die Kulturformen.

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